ABSTRACT

The present invention describes rapid and efficient methods to screen for biomolecular interactions *in vivo* based on protein fragment complementation assays (PCA). Examples are given that demonstrate the utility of the invention and the specific advantages of PCA that are not met by other library screening methods. In a first example, we demonstrate an *in vivo* library-*versus*-library screening strategy that has numerous applications in the identification of novel protein-protein interactions and in directed evolution. In another example we demonstrate the detection of protein-protein interactions starting with defined (full-length) cDNAs, and the concomitant generation of functional assays that provide initial validation of the cDNA products as being biologically relevant. In yet another example we demonstrate cDNA library screening in mammalian cells using a bait-vs.-library strategy combined with fluorescence detection. In a further example we systematically screened a large cDNA collection using automated PCA, combined with quantitative detection of protein-protein complexes. We show that the invention enables bait-vs.-library, library-vs.-library and defined gene screening in any type of cell or cellular context, and using a wide range of reporters and detection methods. The invention allows for identifying and validating genes involved in any cellular process and also provide ready-made assays to study effects of potential drugs, proteins or gene knockouts on specific pathways.